

CLAIMS:

1. A microbial composition comprising a synergistic mixture of at least following two bacterial strains present in about equal proportion for the neutralization of alkaline waste-waters:

a. *Bacillus alkalophilus*

b. *Bacillus sp.*

2. A process for the preparation of microbial composition useful for the neutralization of alkaline waste-waters, which comprises:

(a) isolating the bacterial strains *Bacillus alkalophilus* and *Bacillus sp.* from sewage by standard method;

(b) inoculating the said individual bacterial strains in an Alkaline *Bacillus* broth medium containing $MgSO_4$;

(c) growing the said individual bacterial strains for 16-24 hrs;

(d) inoculating the said individual bacterial strains from step (c) in a modified Tryptone Soya Broth medium, having original pH value about 7.0, the said pH value of the medium being adjusted to different pH values ranging between 8.0-11.0, using Tris-HCl buffer and NaOH- Na_2CO_3 buffer or $NaHCO_3$ - Na_2CO_3 buffer under sterile conditions:

(e) growing the said individual bacterial strains obtained from step (d) in modified Tryptone Soya Broth medium for 16-24 hrs. at different pH values ranging from 8.0-11.0 to selected the 4 bacterial strains growing at pH 11.0 and the

remaining bacterial strains unable to grow at pH 11.0 being acclimatized at pH 11.0.

(f) inoculating the said selected acclimatized individual bacterial strains from step (e) in a modified Nutrient Broth medium, having pH values about pH 7.0, the said pH being adjusted to different pH values ranging from 9.0 -11.0 using $\text{NaOH-Na}_2\text{CO}_3$ buffer or $\text{NaHCO}_3\text{-Na}_2\text{CO}_3$ buffer under sterile conditions;

(g) adding a dye, phenol red indicator and optionally 1 % carbohydrate to the said inoculated medium of individual bacterial strains obtained from step (f) to observe the change in colour for acid production and to identify the acid producing strains at pH 9.0 -11.0;

(h) growing the said inoculated bacterial strains obtained from step (g) for a period of at least 2 days and thereafter observing acid production by the change in colour of phenol red in the said medium from red to orange, orange to yellow and by measuring the decrease in pH of the said medium;

(i) selecting the acid producing bacterial strains at pH 11.0;

(j) mixing the said selected bacterial strains from step (I) to obtain mixed bacterial suspension;

(k) centrifuging the mixed suspension of bacterial strains obtained from step (j) at 8,000 -12,000 rpm to obtain pellet;

(l) washing the obtained pellet from step (k) by suspending the pellet in triple distilled water and recentrifuging at 8,000 -12,000 rpm; and

(m) collecting the pellet from step (I) and lyophilizing the obtained pellet to store at 1 to 4oC for longer use;

3. A process as claimed in claim 2 further comprising :

(a) reconstituting the lyophilized bacterial powder obtain in step (m) by adding 50 ml distilled water;

(b) adding 10% reconstituted bacterial strains in alkaline medium and alkaline waste-waters containing phenol red indicator and optionally 1% carbohydrate; and

(c) observing acid production by the change in colour of phenol red in modified Nutrient Broth medium and in the alkaline waste waters from red to orange, orange to yellow and by measuring the decrease in pH of the said medium as well as of alkaline waste-waters to neutral after a period of at least 2 days;

4. A process as claimed in claim 2 wherein in step (j), the acid producing bacterial strains identified are *Bacillus alkalophilus*, *Bacillus sp.*

5. A process as claimed in claim 2 wherein in step (d), the medium used is Tryptone Soya Broth modified by removal of Papaic digest of soyabean meal and changing the amount of ingredients.

6. A process as claimed in claims 2 wherein in step (d), the medium used is Nutrient Broth modified by addition of K_2HPO_4 and changing the amount of ingredients.

7. A method for the neutralization of alkaline waste-waters using a microbial composition, said method comprising adding a synergistic mixture of at least two bacterial strains namely, *Bacillus alkalophilus* and *Bacillus sp.* which are present in about equal proportion, with the alkaline waste-waters.

8. A method as claimed in claim 7 comprising:

(a) reconstituting lyophilized bacterial powder obtain in step (m) of claim 2 by adding 50 ml distilled water;

(b) adding 10% reconstituted bacterial strains in alkaline medium and alkaline waste-waters containing phenol red indicator and optionally 1% carbohydrate; and

(c) observing acid production by the change in colour of phenol red in modified Nutrient Broth medium and in the alkaline waste waters from red to orange, orange to yellow and by measuring the decrease in pH of the said medium as well as of alkaline waste-waters to neutral after a period of at least 2 days;

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